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Autoantibodies Developing to Myeloperoxidase and Proteinase 3 in Systemic Vasculitis Stimulate Neutrophil Cytotoxicity Toward Cultured Endothelial Cells

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The ability of vasculitis-associated anti-neutrophil cytoplasm antibodies (ANCA) to activate neutrophils and mediate release of radiolabel from ^{111}Indium-labeled cultured human umbilical vein endothelial cells (HUVEC) was determined as a measure of the potential cytotoxicity of ANCA-activated neutrophils against vascular endothelium. Priming of neutrophils with low doses of phorbol 12-myristate 13-acetate (PMA) (1 ng/ml) and ionomycin (0.1 μmol/l) was required, together with pretreatment of endothelial cells with BCNU (1,3-bis-{2-choroethyl}-1-nitrosurea; 0.26 mmol/l). Under these conditions and using a 4-hour serum-free assay system, mouse monoclonal antibodies (MAb) to the target autoantigens proteinase-3 (Pr-3) and myeloperoxidase (MPO) mediated enhanced release of ^{111}Indium from HUVEC compared with control MAb. Human IgG Fab$_2$ C-ANCA (recognizing Pr-3) and P-ANCA (recognizing MPO) did likewise. Preactivation of HUVEC with TNF (50 U/ml, 4 hr) enhanced the release of ^{111}Indium from HUVEC generated by neutrophils activated with anti-Pr-3 and anti-MPO MAb. These data support the suggestion that activation of neutrophils by ANCA within the vascular lumen may contribute to endothelial cell injury. (Am J Pathol 1992, 141: 335–342)

The presence of autoantibodies to neutrophil and monocyte cytoplasmic components is closely related to the development of primary idiopathic systemic vasculitis syndromes including the granulomatoses (Wegener's granulomatosis and the Churg Strauss syndrome) and the polyarteritis group (classical polyarteritis nodosa, microscopic polyarteritis, and its renal-limited variant idiopathic rapidly progressive glomerulonephritis). The antineutrophil cytoplasm antibodies (ANCA) recognize at least two neutrophil primary granule constituents, namely proteinase-3 (Pr-3) and myeloperoxidase (MPO), which may be broadly distinguished by the pattern of indirect immunofluorescence staining of ethanol-fixed normal neutrophils: anti-Pr-3 antibodies give a granular cytoplasmic stain (classical or C-ANCA) whereas anti-MPO antibodies give a perinuclear pattern (P-ANCA).[1] Both C-ANCA and P-ANCA antibodies can activate neutrophils *in vitro* to cause a respiratory burst and to mediate degranulation.[2] The histopathologic findings in systemic vasculitis disorders such as Wegener's granulomatosis and polyarteritis suggest that the endothelium of blood vessel walls is a major target for injury.[3] We undertook studies to determine whether ANCA-activated neutrophils can injure vascular endothelial cells in culture.

Materials and Methods

Reagents and Antibody Preparations

Anti-MPO MAb (IgG1, purified MAb 150 μg/ml) was purchased from Dako (Dako Ltd., High Wycombe, Bucks, England). Anti-Pr-3 MAb (IgG1, ascites preparation 5 mg/ml of IgG) was a gift from Roel Goldschmeding, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam. Control MAbs of the same isotype were used as appropriate. Human

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IgG Fab_2 preparations were made from serum obtained from normal individuals, from a patient with anti-glomerular basement membrane antibody mediated glomerulonephritis, and from three patients with systemic vasculitis associated with P-ANCA (one patient) or with C-ANCA (two patients), respectively, and whose serum did not contain anti-endothelial cell antibodies.[4] The P-ANCA and C-ANCA sera showed perinuclear and granular cytoplasmic patterns of staining, respectively, and by ELISA were reactive with purified MPO (P-ANCA) and Pr-3 (C-ANCA). Antibody preparations diluted to the highest concentrations used in the experiments were tested for endotoxin using a limulus amoebocyte lysate assay (E-toxate assay; Sigma Chemical Co., Poole, Dorset, England), and levels were found to be less than 0.06 EU/ml.

Culture of HUVEC

Endothelial cells were isolated from human umbilical cords as described by Jaffe[5] and serially passaged as described by Thornton et al.[6] Cells between subcultures two and four were used for cytotoxicity assays.

Isolation of Neutrophils

Neutrophils were isolated as previously described.[7] Briefly, 50 ml of freshly drawn blood from healthy volunteers was anticoagulated with 5 ml of 4.2% disodium hydrogen citrate and 5% glucose and mixed with an equal volume of 2.5% hydroxyethyl starch (Hespan, Du Pont Ltd., Stevenage, Herts, England). Red cells were sedimented by gentle centrifugation at 20*g* for 10 minutes. Leukocyte-rich plasma was then removed, and leukocytes were pelleted by centrifugation at 150*g* for 7 minutes. The platelet-rich plasma supernatant was recovered and platelet-poor plasma was obtained by two successive centrifugation procedures at 1500*g* for 10 minutes. Leukocytes were resuspended in equal volumes of plasma and 55% percoll (Pharmacia LKB Biotechnology, Uppsala, Sweden) in sterile calcium and magnesium-free phosphate-buffered saline and applied to the top of a discontinuous density gradient consisting of 81% percoll over 70% percoll. The gradient was centrifuged at 1600*g* for 25 minutes. Neutrophils were obtained from the 81%/70% percoll interface, washed twice with platelet-poor plasma and twice with HEPES-buffered Tyrodes solution containing 0.1% bovine serum albumin (BSA, fraction V, Sigma Chemical Co., Poole, Dorset, England), pH 7.4. Neutrophils (5×10^6/ml) were treated with cytochalasin B (5 μg/ml; Sigma Chemical Co., Poole, Dorset, England) for 5 minutes before use to inhibit actin polymerization and permit degranulation. Neutrophils isolated by this method were greater than 98% pure after staining with Giemsa and more than 99% viable as assessed by Trypan blue exclusion. All steps were performed at room temperature.

Indium-labeling and BCNU or TNF Treatment of HUVEC

Confluent monolayers of HUVEC in 96-well flat-bottom trays were washed twice with Hank's balanced salt solution (HBSS) containing calcium and magnesium and then incubated with 1 μCi/well of indium chloride (Amersham International plc, Amersham, England) coupled to 1-hydroxypyridine 2-thione sodium salt (Sigma Chemical Co., Poole, Dorset, England) diluted in 100 μl of HBSS at 37°C for 15 minutes. Cells were then washed twice with RPMI 1640 (Gibco Ltd., Uxbridge, Middlesex, England) containing 4% BSA (RPMI/BSA). After a 60-minute incubation in RPMI/BSA, HUVEC were treated with 0.26 mmol/l BCNU (1,3-bis-{2-choroethyl}-1-nitrosurea; Bristol-Myers, Uxbridge, Middlesex, England) in RPMI/BSA for a further 15 minutes at 37°C.[8] BCNU has a variety of effects on proliferating cells such as inactivation of enzymes including glutathione reductase,[8] thereby reducing the ability of the cells to withstand oxidative damage. After 15 minutes, HUVEC were washed twice with HBSS and used for the cytotoxicity assay.

For some experiments, HUVEC were treated with tumor necrosis factor (TNF; Genzyme, specific activity 2×10^7 units/ml) at 50 U/ml for 4 hours before labeling with indium.

Cytotoxicity Assays

^{111}Indium-labeled HUVEC (1.2×10^4/well) were coincubated with 5.0×10^5 neutrophils/well in HBSS. Phorbol 12-myristate 13-acetate (PMA; Sigma Chemical Co., Poole, Dorset, England) and ionomycin (Calbiochem Corp., San Diego, CA) were added at the concentrations indicated, as were MAb or human $F(ab)_2$ preparations. Spontaneous release of radioactivity was assessed by wells that contained HUVEC and HBSS only. Maximum possible release of radioactivity was assessed by lysing HUVEC with Nonidet P-40 2% (Sigma Chemical Co., Poole, Dorset, England). Reagents were added to HUVEC in the following order: HBSS, ionomycin, neutrophils, PMA, antibodies. In preliminary experiments, the findings were similar either when PMA and ionomycin were present in the neutrophil-HUVEC cocultures throughout,

or when neutrophils were primed with PMA and ionomycin for 10 minutes, washed, and then cocultured with HUVEC. After the addition of reagents and cells to the wells, the plates were centrifuged for 2 minutes at 20*g* and then incubated at 37°C for 4 hours. A 4-hour incubation period was used since in preliminary time-course experiments this gave the best differentiation between test and control samples. After 4 hours, plates were centrifuged for 5 minutes at 200*g*, then 100 μl of each supernatant was transferred to a tube for counting in a Pharmacia LKB 1250 multigamma counter. The percentage release of indium was calculated from the equation:

$$100 \times \left[\frac{\text{release from test sample} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \right]$$

Tests were performed in triplicate or quadruplicate on any one plate and results were analyzed using a Mann-Whitney U-test.

Results

Neutrophils Activated with PMA and Ionomycin Lyse BCNU-treated HUVEC

Neutrophils activated by PMA (>1 ng/ml) caused increased release of ^{111}Indium from BCNU-treated HUVEC, whereas neutrophils treated with ionomycin (up to 1 μmol/l) showed no or small increases in the release of ^{111}Indium (Figure 1). However, treatment with ionomycin (0.01–1μM) of neutrophils that had been primed with a low dose of PMA (1 ng/ml) led to a greater than additive release of radioactivity from BCNU-HUVEC, particularly at 1 μmol/l. Thus in further assays, the effects of antibodies to neutrophil components were assessed using low priming doses of PMA (1 ng/ml) and/or ionomycin (0.1 μmol/l) since at these concentrations neutrophils showed low levels of intrinsic cytotoxicity towards HUVEC. BCNU treatment of HUVEC was not essential for lysis but the percentage release of radioactivity was smaller in its absence and only just discernible at high concentrations of PMA (100 ng/ml) (Table 1).

Primed Neutrophils Can Be Activated by Anti-MPO MAb to Lyse HUVEC

The ability of anti-MPO MAb alone (at concentrations up to 40 μg/ml) to activate unprimed neutrophils to lyse BCNU-treated HUVEC was variable between experiments but was rarely more than 5% above control antibody levels. In contrast, anti-MPO MAbs at concentrations as low as 0.01 μg/ml were able to activate neutrophils that had been primed with PMA (1 ng/ml) and ionomycin (0.1 μmol/l) (Figure 2a). In other experiments anti-MPO was tested in concentrations up to 40 μg/ml and percentage release of ^{111}Indium was up to 28.3%. Use of one priming agent only, at the doses indicated, together with anti-MPO MAbs did not consistently activate neutrophils.

Primed Neutrophils Can Be Activated by Anti-Pr-3 MAb to Lyse HUVEC

Anti-Pr-3 antibodies (up to 40 μg/ml) were not able to activate unprimed neutrophils (less than 5% release of indium) to lyse BCNU-treated HUVEC, but anti-Pr-3 MAbs were effective at activating PMA (1 ng/ml) and ionomycin (o.1 μmol/l) primed neutrophils (Figure 2b). Anti-Pr-3 was tested in concentrations up to 40 μg/ml and at this concentration mediated up to 17.3% release of ^{111}Indium from HUVEC.

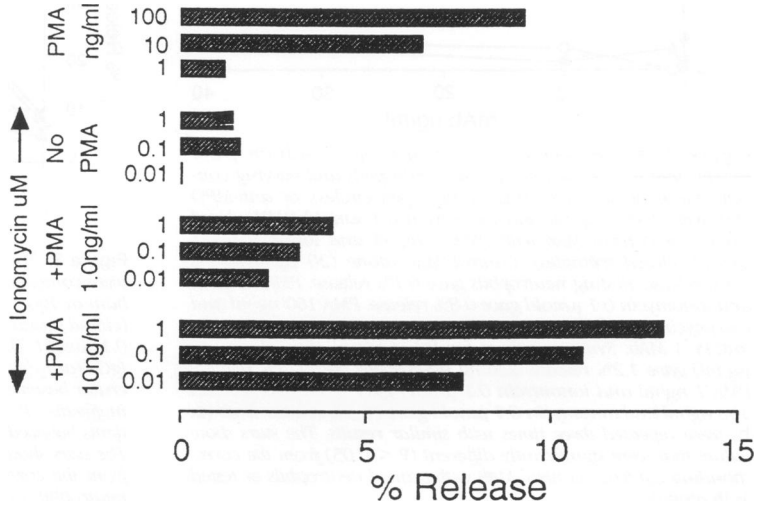

Figure 1. *The percentage release of ^{111}indium from HUVEC that were coincubated with neutrophils and varying concentrations of PMA and/or ionomycin. The release of ^{111}indium in the presence of resting neutrophils alone, PMA alone (all doses), or ionomycin alone (all doses) was less than 1.0% in each case. The standard errors of the means were less than 0.5%. The experiment was repeated four times with similar results.*

Table 1. *Release of Indium from BCNU-treated or Untreated HUVEC by Resting or PMA (100 ng/ml)-activated Neutrophils*

	HUVEC treatment	% Release of indium after coincubation with	
		Resting neutrophils	PMA-activated neutrophils
Exp 1.	None	0	0.3
	BCNU	6.1	15.2
Exp 2.	None	0.7	2.9
	BCNU	8.6	21.4

Primed Neutrophils Can Be Activated by P-ANCA or C-ANCA Fab_2 to Lyse HUVEC

Both P-ANCA and C-ANCA IgG Fab_2 (at concentrations of 0.1 mg/ml) increased lysis of BCNU-treated HUVEC by activating PMA (1 ng/ml) and ionomycin (0.1 μmol/l) primed neutrophils (Figure 3). One P-ANCA and two C-ANCA IgG Fab_2 preparations were tested with similar results. IgG Fab_2 from pooled normal human IgG or from a patient with antiglomerular basement membrane glomerulonephritis were without effect.

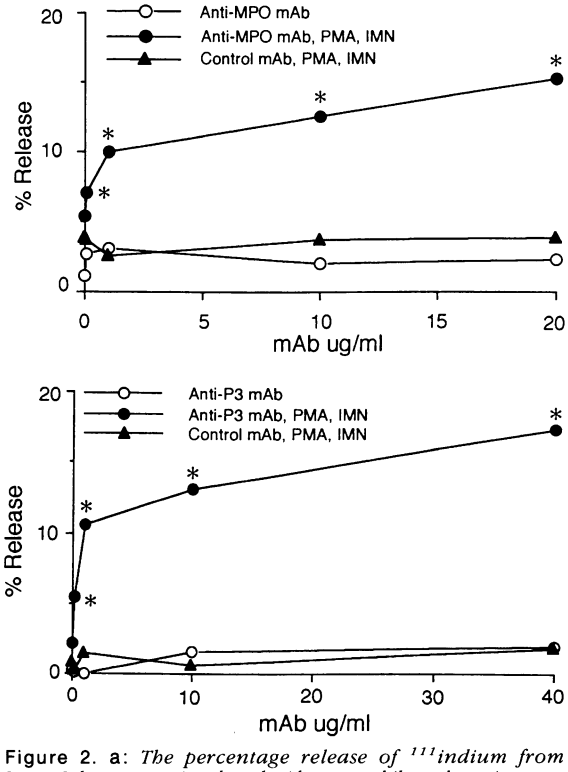

Figure 2. a: *The percentage release of ^{111}indium from HUVEC that were coincubated with neutrophils and varying concentrations of anti-MPO MAb alone (open circles), or anti-MPO MAb with PMA 1 ng/ml and ionomycin 0.1 μmol/l (IMN; closed circles) or control MAb with PMA 1 ng/ml and ionomycin 0.1 μmol/l (closed triangles). Control MAb alone (20 μg/ml) gave 3.8% release. Resting neutrophils gave 0.1% release. PMA 1 ng/ml and ionomycin 0.1 μmol/l gave 0.8% release. PMA 100 ng/ml and ionomycin 0.1 μmol/l gave 29.3% release mol/l.* **(b)** *shows data for anti-Pr-3 MAb. Symbols are as for* **(a).** *Control MAb alone (40 μg/ml) gave 1.2% release. Resting neutrophils gave 0.4% release. PMA 1 ng/ml and ionomycin 0.1 μmol/l gave 0.8% release. PMA 100 ng/ml and ionomycin 0.1 μmol/l gave 16.5% release. Both* **(a, b)** *were repeated three times with similar results. The stars show values that were significantly different ($P < 0.05$) from the corresponding controls (control MAb with primed neutrophils or tested MAb alone).*

HUVEC Activation by TNF Promotes Lysis by Anti-MPO or Anti-Pr-3 MAb

To determine whether HUVEC were more susceptible to lysis after cytokine activation, the HUVEC were pretreated with 50 U/ml of TNF for 4 hours before exposure to anti-Pr-3 or anti-MPO activated neutrophils. The TNF was washed away before coculture of neutrophils with HUVEC. Cytokine-activated BCNU-treated HUVEC were more susceptible to lysis by both unprimed and primed (PMA 1 ng/ml; ionomycin 0.1 μmol/l) neutrophils that were activated with anti-Pr-3 MAb or with anti-MPO MAb (Figure 4).

The ability of neutrophils to lyse TNF-activated HUVEC that had not been treated with BCNU was also studied. There was little release of indium (<2.0%) from unactivated HUVEC even when the neutrophils were treated with priming doses of PMA (1 ng/ml) and ionomycin (0.1 μmol/l), TNF (50 U/ml), and anti-MPO MAb (40 μg/ml). Primed neutrophils released low levels of indium from TNF-activated HUVEC, equivalent in order of magnitude (5–8% release) to that seen with primed neutrophils and BCNU-treated HUVEC (Figure 5). Further activation of neutrophils with either TNF (50 U/ml) or with anti-MPO MAb (40 μg/ml) caused a further increase in the release of indium, whereas activation of neutrophils

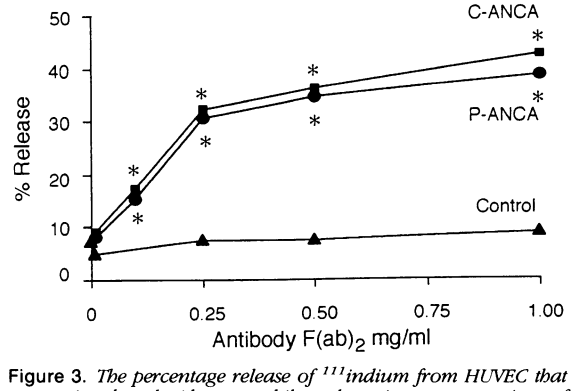

Figure 3. *The percentage release of ^{111}indium from HUVEC that were coincubated with neutrophils and varying concentrations of human IgG Fab_2 containing P-ANCA (closed circle) or C-ANCA (closed square) activity, together with PMA 1 ng/ml and ionomycin 0.1 μmol/l. The control antibody shown here was pooled normal IgG $F(ab)_2$ (triangle) but IgG $F(ab)_2$ from a patient with antiglomerular basement membrane antibody mediated nephritis was also ineffective. P-ANCA from one patient and C-ANCA from two patients behaved similarly. The experiment was repeated three times. The stars show values that were significantly different ($P < 0.05$) from the corresponding controls (control antibody with primed neutrophils or test antibody alone).*

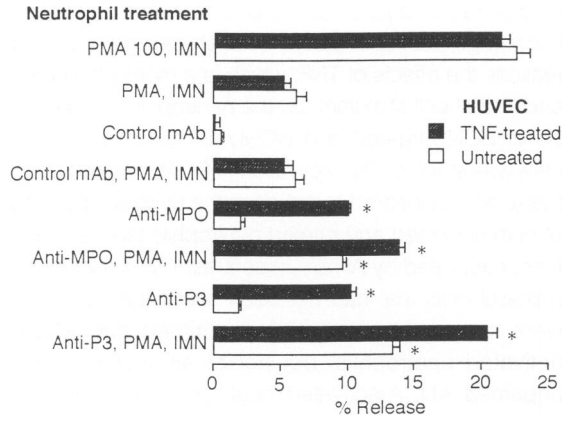

Figure 4. *The percentage release of ^{111}indium from BCNU-pretreated HUVEC that were unactivated or preactivated with TNF (50 U/ml for 4 hr) and coincubated with neutrophils activated with various combinations of PMA 1.0 ng/ml (100 ng/ml in top bars), ionomycin (IMN) 0.1 μmol/l, and control or anti-MPO or anti-Pr-3 MAbs 10 μg/ml. Percent release with resting neutrophils was 0.4% with untreated HUVEC, 1.1% with TNF-treated HUVEC. The experiment was repeated three times. The bars denote the standard errors of the mean of three observations. The stars show values that were significantly different (P < 0.05) from the appropriate controls (PMA and ionomycin primed neutrophils with either unactivated or TNF-activated HUVEC).*

using all four reagents caused the greatest release of indium from the TNF-activated HUVEC (13.4% in Figure 5). Treatment of neutrophils with TNF (50 U/ml) and anti-MPO MAb (40 μg/ml) only, mediated release of indium that was significantly different (P = 0.05) from the basal release that occurred during coculture of resting neutrophils and HUVEC but was less than that seen with primed (PMA 1.0 ng/ml; ionomycin 0.1 μmol/l) neutrophils.

Discussion

Systemic vasculitis is characterized by the development of autoantibodies to endothelial cells and to leukocyte enzymes on the one hand, and by presence of fibrinoid necrosis and inflammation of blood vessel walls on the other. These associations raise the possibility that the development of autoantibodies is causally related to the vascular damage. Evidence that anti-endothelial cell antibodies are involved in the primary pathogenesis of vasculitic lesions is weak.[4] However there is a close association between the presence of ANCA and the development of vasculitis, as well as between the presence of ANCA and disease relapse.[9,10] We report that mouse monoclonal antibodies or human autoantibodies to MPO or Pr-3 are capable of further activating primed neutrophils to mediate lysis of cultured endothelial cells, measured by the release of ^{111}Indium.

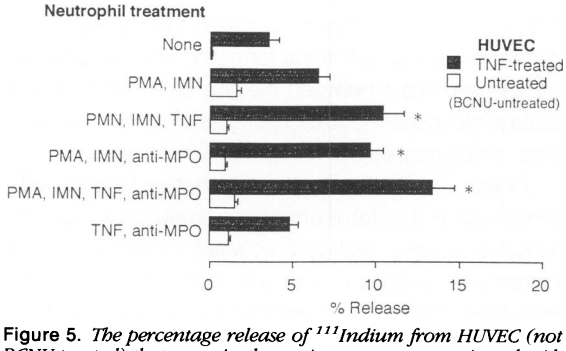

Figure 5. *The percentage release of ^{111}Indium from HUVEC (not BCNU-treated) that were in the resting state or preactivated with TNF (50 U/ml for 4 hr) and then coincubated with neutrophils that had been stimulated with various combinations of PMA 1.0 ng/ml, ionomycin (IMN) 0.1 μmol/l, TNF (50 U/ml) and anti-MPO MAb (40 μg/ml). Control MAb, TNF, or anti-MPO MAb alone caused less than 1% release of indium from resting HUVEC and between 2–3.2% release from TNF-activated HUVEC. The experiment was repeated twice. The bars denote the standard errors of the mean of three observations. The stars show values that were significantly different (P < 0.05) from the control (PMA and ionomycin primed neutrophils with TNF-activated HUVEC).*

In the present studies, the neutrophils were primed with low levels of PMA and ionomycin to obtain significant damage to BCNU-treated HUVEC by ANCA-activated neutrophils. This was unexpected since earlier observations by Falk et al[2] suggested that ANCA alone were stimulatory to neutrophils. There are two possible explanations for the requirement for neutrophil priming in our model. The first is that greater release of reactive oxygen species and/or enzymes may be required to damage endothelial cells than is produced by neutrophils that have been prepared according to Falk et al.[2] That this may be so is suggested by more recent preliminary studies reported by Falk and coworkers concerning endothelial damage by neutrophils, which showed that ANCA alone is insufficient and that TNF is also required to stimulate the neutrophils.[11] The second explanation concerns the method of preparation of neutrophils in the two laboratories. Falk et al used heparin-treated blood and plasmagel plus histopaque to separate the neutrophils,[12] whereas this study used sodium citrate-treated blood and plasma Percoll discontinuous gradient centrifugation.[7,13] The latter method has been shown to result in less stimulation of neutrophils[13] and may be a major reason why we needed to prime the neutrophils in our model.

We chose to prime the neutrophils in our model with PMA and ionomycin for two reasons. First, the effects of these agents on signal transduction pathways have been extensively studied (PMA activates protein kinase C, whereas ionomycin induces a calcium flux). Second, PMA and ionomycin have been used in models of neutrophil killing of nonendothelial cell targets, which have shown that activation can be more effectively achieved with multiple stimulants that use different signal transduction pathways.[14,15] For example, lysis of the erythroleukemia K562 cell line by PMA-stimulated neutrophils is potentiated by the calcium ionophore, ionomycin.[14] The synergism between PMA and ionophore was strongest at subthreshold doses of PMA, suggesting that dual signals (mediated by PMA-induced protein kinase C activation

and by ionomycin-induced changes in intracellular calcium) are required for the maximal expression of lytic function. In preliminary studies, we confirmed these synergistic effects of PMA and ionomycin on neutrophil-mediated lysis of K562 cells (data not shown). We had hoped that ANCA might partner with one or other priming agent to activate neutrophils to lyse endothelial cells, thereby giving additional information as to the mode of action of ANCA at the signal transduction level. In the studies reported, neutrophil activation by antibodies to MPO and Pr-3 was enhanced after treatment of the neutrophils with low doses of PMA (1ng/ml) and ionomycin (0.1 μmol/l). Combined use of both PMA and ionomycin for priming was more consistently effective than either agent alone, and a clear preference for one or other priming agent was not demonstrated (data not shown). However, we are aware that others have suggested that ANCA may activate neutrophil protein kinase C[16,17] and further studies are required to determine the relationship between these findings and our own. The main function of the priming agent may be to mediate translocation of the ANCA antigens to the cell surface where they become available for binding by ANCA, rather than by acting synergistically with ANCA during signal transduction to augment release of reactive oxygen species and/or enzymes.

In the present studies, the cultured endothelial cells were routinely treated with BCNU, which inhibits glutathione reductase, thus reducing the ability of the cells to withstand oxidant injury.[8,18] Others have shown that human endothelial cells derived from umbilical veins are resistant to the injurious effects of activated neutrophils but that the potential for injury by PMA-activated (100 ng/ml) neutrophils may be revealed if the capacity of the HUVEC to resist injury is reduced by treatment with BCNU; unstimulated neutrophils and PMA alone are not toxic to HUVEC, whereas PMA-activated neutrophils are not toxic to BCNU-untreated HUVEC.[19] Our work confirms these findings. Endothelial cells derived from different species or from different sites within the same species also vary in their susceptibility to neutrophil-mediated injury.[20,21] Furthermore, endothelial cells from different sites may vary in their susceptibility to injury by different secreted components such as reactive oxygen metabolites or proteolytic enzymes from activated neutrophils, and HUVEC may not be truly representative of microvascular endothelial cells in this regard.[21] To maximize the likelihood of demonstrating cytotoxic damage to HUVEC by ANCA-activated neutrophils, the HUVEC were pretreated with BCNU. In patients with systemic vasculitis, the endothelial cells at those sites that are frequently affected by vasculitic inflammatory lesions, for example the glomeruli, may be inherently less capable of withstanding neutrophil-induced injury or their viability may be reduced for other reasons. Nevertheless, we acknowledge that use of BCNU is not physiologic. For these reasons the effects of TNF, which is a more physiologic endothelial cell stimulant, on the neutrophil-mediated lysis of BCNU-treated and BCNU-untreated endothelial cells was studied. TNF activation of BCNU-treated endothelial cells appeared to enhance the cytotoxic potential of both unprimed and primed neutrophils that had been further activated by ANCA. In contrast TNF activated endothelial cells that had not been sensitized by BCNU, were somewhat susceptible to primed and ANCA-activated neutrophils, but not at all susceptible to unprimed ANCA-activated neutrophils. The ability of ANCA-activated neutrophils to lyse TNF-activated, BCNU-untreated endothelial cells varied depending on the priming agent used; at the concentrations at which the priming agents were used here, the combined use of three agents (PMA, ionomycin, and TNF) was more effective than two agents (PMA and ionomycin) which, in turn, was more effective than TNF alone. These data suggest that less stimulation of neutrophils is required to mediate damage to endothelial cells whose ability to withstand injury has been reduced for whatever reason. Further, the agents used for priming and activating the neutrophils also dictate the degree of injury. TNF is a physiologic activator of both endothelial cells and neutrophils and levels are raised in patients with systemic vasculitis.[22] TNF has multiple effects on endothelial cells[23] including the stimulated expression of leukocyte adhesion molecules such as endothelial leukocyte adhesion molecule-1 (ELAM-1), which is recognized by the sialyated Lewis X ligand on the neutrophil surface[24] and intercellular adhesion molecule-1 (ICAM-1), which is recognized by the CD11/CD18 complex on the neutrophil.[25] Neutrophil activation by TNF is enhanced also when the neutrophils are adherent to a surface via CD11/CD18.[26,27] Close adhesion between the neutrophil and its target cell may allow the formation of a sequestered microenvironment between the two cells, which is less accessible to the modulating effects of proteinase inhibitors, which might inactivate proteolytic enzymes.[28]

Finally, it is worth considering whether lysis of endothelial cells is a suitable endpoint from which to consider ANCA-stimulated neutrophil activities. Lysis may be one extreme of a series of injurious effects that may befall endothelial cells that are exposed to products secreted by fully activated neutrophils on the luminal side of the endothelium. There is evidence that neutrophil products are capable of altering signal transduction and linked metabolic events in endothelial cells. For example, hydrogen peroxide may interfere with the thrombin-stimulated release of prostacyclin and platelet-activating factor from the endothelial cell.[29] We have not specifically looked for evidence of altered endothelial function in the presence

of ANCA-stimulated neutrophil activation, but such effects could contribute to development of the vasculitic lesions.

In summary, vascular injury in ANCA-associated vasculitic disorders may be triggered by the autoantibodies, which may directly activate neutrophils as suggested.[2] However, the presence of autoantibodies and neutrophils may be necessary but not sufficient for overt injury. Other factors that may be required include the presence of agents that prime neutrophils (such as TNF or endotoxin), adherence of the neutrophil to the endothelial wall, and possibly reduced ability of the endothelial cells to resist neutrophil-mediated enzymic or oxidative injury.

Acknowledgments

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